

Stem Cells: Emerging Therapeutic Aids in Dental and Maxillofacial Surgery: A Review

¹Mishra Abhishek, ²Maria Anisha, ³Tiwari Mala

¹PG Student oral and maxillofacial surgery, Rishiraj College of Dental Sciences Bhopal (MP), India

Abstract: Recent exciting discoveries place dentists at the forefront of engaging their patients in potentially life-saving therapies derived from a patient's own stem cells located in deciduous and permanent teeth. With appropriate biochemical signals stem cells can be transformed into desirable cells. The idea behind this article is to shortly review the obtained literature on stem cell with respect to their properties, types and advantages of dental stem cells. Emphasis has been given to the possibilities of stem cell therapy in the oral and maxillofacial region including regeneration of tooth and craniofacial defects.

Keywords: Cryopreservation, Dental follicle embryonic stem cells, Pluripotent stem cells, Periodontal and Craniofacial defect.

I. INTRODUCTION

Recent exciting discoveries place dentists at the forefront of engaging their patients in potentially life-saving therapies derived from a patient's own stem cells located in deciduous and permanent teeth. In 2000, the National Institutes of Health (NIH) released two studies of research on human teeth detailing the discovery of adult stem cells in impacted third molars and even more resilient stem cells in deciduous teeth¹.

Replacement of oromaxillofacial structure is difficult, because functions such as facial expression, articulation, chewing, and swallowing are delicate and made of a complex anatomical structure formed from soft and hard tissues. Stem cells, biomimetic materials, and growth factors are essential to form these three-dimensional structures. Regeneration of oral and maxillofacial structures can be carried out using stem cell therapy that has gained momentum in the recent days².

II. BODY OF ARTICLE

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce extra stem cells. They are originated in multicellular organisms. Research into stem cells raised out of findings by Ernest A. McCulloch and James E. Till (1960s) at the University of Toronto. In 2005 Dr. Irina Kerkis discovered dental pulp stem cells (DPSCs). Later on researchers have recognized the mesenchymal type of stem cell inside dental pulp. This type of stem cells has the future potential to distinguish into a various type of other cells.

Stem cell types³:

Stem cells can be broadly divided into

1. Embryonic stem cell
2. Adult stem cell
 - Hematopoietic stem cell
 - Mesenchymal stem cell

3. Induced pluripotent stem cell
4. Amniotic fluid derived stem cell
5. Umbilical cord blood stem cell
6. Bone marrow derived stem cells

Embryonic stem cells:

Embryonic stem cells (ESCs) are derived from the cells of the inner cell mass of the blastocyst during embryonic development. ESCs have the capacity to differentiate into any cell type and the ability to self-replicate for numerous generations. A potential disadvantage of ESCs is their ability to differentiate into any cell lineage and to proliferate endlessly unless controlled. The clinically observed teratoma is a tumor that is an example of ESCs growing into a “different and undesired tissue.” ESCs can be obtained only from embryos, and therefore are associated with ethical issues⁴.

Adult stem cells:

Sources of adult stem cells include the umbilical cord, amniotic fluid, bone marrow, adipose tissue, brain and teeth.^{5,6} Adult stem cells are not subject to the ethical controversy that is associated with embryonic stem cells; they can also be autologous and isolated from the patient being treated, whereas embryonic stem cells cannot.

Induced pluripotent stem cells (iPS):

The newly discovered iPS cells are adult or somatic stem cells that have been coaxed to behave like embryonic stem cells. iPS cells have the capacity to generate a large quantity of stem cells as an autologous source that can be used to regenerate patient-specific tissues. However, even the authors of these recent reports have cautioned that any carcinogenic potential of iPS cells should be fully investigated before any commercialization can be realized⁶.

Amniotic fluid-derived stem cells (AFSCs):

AFSCs can be isolated from aspirates of amniocentesis during genetic screening. An increasing number of studies have demonstrated that AFSCs have the capacity for remarkable proliferation and differentiation into multiple lineages such as chondrocytes (for cartilage), adipocytes (for fat), osteoblasts (for bone), myocytes (for muscle), endothelial cells, neuronlike cells and live cells.^{7,8,9} The potential therapeutic value of AFSCs remains to be discovered.

Umbilical cord blood stem cells (UCBSCs):

UCBSCs derive from the blood of the umbilical cord.¹⁸ There is a growing interest in their capacity for self-replication and multilineage differentiation, and UCBSCs have been differentiated into several cell types that resemble cells of the liver, skeletal muscle, neural tissue, pancreatic cells, immune cells and mesenchymal stem cells.^{10,11,12,13} Several studies have shown the differentiation potential of human UCBSCs in

treating cardiac²⁵ and diabetic diseases in mice.¹⁴ The disadvantage of UCBSCs is that there is only one opportunity to harvest them from the umbilical cord at the time of birth. Similarly, amniotic stem cells can be sourced only from amniotic fluid and are therefore subject to time constraints.

Bone marrow-derived stem cells (BMSCs):

BMSCs consist of both hematopoietic stem cells that generate all types of blood cells and stromal cells (mesenchymal stem cells) that generate bone, cartilage, other connective tissues and fat. BMSCs are currently the most common commercially available stem cell.¹⁵ They can be isolated from bone marrow aspiration or from the collection of peripheral blood-derived stem cells following chemical stimulation of the bone marrow, by means of subcutaneous injection, to release stem cells.

STEM CELLS FROM THE ORAL AND MAXILLOFACIAL REGION:

Stem cells from oral and maxillofacial region predominantly contain mesenchymal stem cells. In oral and maxillofacial area different types of dental stem cells were isolated and characterized. They include

- Dental pulp stem cells (DPSCs)¹⁶
- Stem cells from exfoliated deciduous teeth (SHED)¹⁷
- Periodontal ligament stem cells (PDLSCs)¹⁸
- Stem cells from apical papilla (SCAP)¹⁹
- Dental follicle progenitor cells (DFPCs)²⁰

Stem Cell Handling and Cryopreservation:

Stem cells are released from small amounts of tissue, in the case of dental stem cells from dental pulp. The tissue is placed in an enzyme solution that releases the stem cells, which are then cultured to multiply. This can be accomplished using serum-free medium, removing the need for use of animal serum. Differentiation then occurs and the cells are transplanted – either alone or with a scaffold or other biomaterials, depending on the application.

Cryopreservation:

Stem cells must be derived from living tissue and must be preserved. This is achieved by cryopreservation. The cells are rapidly cooled to subzero temperatures as low as -196° Celsius, stopping any cellular or biochemical activity. Rapid freezing is necessary to prevent ice from forming around or inside the cells and to prevent dehydration, as these would cause cell damage and death. Extracted permanent and deciduous (including exfoliating) teeth can be preserved for future use with cryopreservation. Research has demonstrated that stem cells derived from the dental pulp of extracted third molars retain the ability to differentiate into multiple cell types following thawing after cryopreservation using liquid nitrogen.²¹ Stem cells derived from the periodontal ligament are viable following cryopreservation.²² After two years of cryopreservation, stem cells have been able to differentiate and to proliferate, and it has been concluded that DSCs can undergo long-term cryopreservation

STEM CELL MARKERS AND SCAFFOLD:

Cultured stem cells should be passed through stem cell markers like Oct4, Nanog, SSEA4, TRA-1-60 and TRA-1-81 before it is administered to patients to know the lineage of the cell. Compulsory endotoxin test should be subjected to the cultured stem cells to rule out any microbial contamination. Stem cells are loaded in an appropriate carrier called “scaffold” to close the defects or replace the organ. Scaffold can be of different shapes, pattern and biomaterials. Depending upon the necessity it can be made up of natural or artificial materials and can be biodegradable or non biodegradable. Materials such as poly lactic acid, polyglycolic acid (PGA), polyethylene terephthalate, polypropylene fumarate, hydroxyapatite/tricalcium phosphate, fibrin, alginates, and collagen are used.²⁴

CLINICAL APPLICATION OF STEM CELL THERAPY IN THE ORO-MAXILLOFACIAL REGION:

The structures of interest in oral and maxillofacial region include the enamel, dentin, dental pulp, cementum, periodontal ligament, craniofacial bones, the temporo mandibular joint, ligaments, skeletal muscles, tendons, skin, subcutaneous soft tissue, and salivary glands.

Regeneration of dentin, pulp:

Dental pulp tissue has the regenerative potential to form dentin in response to any injury.²⁵ Tubular dentin formation was observed when human pulp stem cells with scaffold (hydroxyapatite/tricalcium phosphate) were implanted in immunocompromised mice.²⁶ Reparative dentin formation on amputated pulp was found when stem cells were combined with recombinant human bone morphogenetic protein 2 (BMP 2) in experimental studies on animal models.²⁷

Regeneration of the pulp inside the damaged tooth can be the basic clinical application of stem therapy in dentistry. Root canal treatment in a young permanent molar will stop the tooth's continuous maturation process there by leaving thin egg shell like weak tooth that is susceptible to fracture. Regeneration of pulp with stem cell therapy will be a better option. Stem cells harvested from the pulp of unwanted teeth like third molar can be utilized to regenerate the pulp of severely injured tooth there by preventing the need for endodontic treatment in adults.

Huang²⁸ *et al.* in his review article summarized new protocol for endodontically involved immature permanent teeth in which minimal instrumentation was done in it followed by disinfection with triple antibiotic paste. Treated tooth is coated with mineral trioxide aggregate (MTA) and filled with glass ionomer cement. Periodical observation was done to ascertain root maturation.

Stem cells in periodontal regeneration:

Stem cells will be a promising tool for regenerating the periodontal structures such as periodontal ligament and other supporting elements. BMSCs have been used by Kawaguchi *et al.* for their capability to regenerate periodontal tissue and repair periodontal defects. BMSCs have the ability to produce alveolar bone, periodontal ligament, and *in vivo* cementum after implantation into the periodontal defects. Thus, it was proved BMSCs provides an alternative source for the treatment of periodontal diseases.²⁹

Autologous mesenchymal stem cells from iliac crest in combination with platelet rich plasma from peripheral blood was used for periodontal regeneration. Significant closure of bone defect and improvement of attachment level was observed after one year follow up. It also showed good healing and regeneration of interdental papilla.³⁰

Nagatomo *et al.* in their experimental studies found that PDL cells having stem cell properties can regenerate periodontium.³¹ Transplantation of PDL derived cells into animal models were shown to regenerate periodontal tissue.³²

Iwata *et al.* harvested and expanded primary canine PDL cells *in vitro* and also made into transplantable constructs containing PGA Scaffold and PDL cell sheets. The transplantable constructs in combination with porous bTCP (b – tricalcium phosphate) induced regeneration of periodontal structures, including alveolar bone, cementum, and periodontal fibers.³³

Liu *et al.* regenerated periodontal tissue in miniature swine using scaffolds seeded with periodontal ligament derived stem cells³⁴. PDLSCs can differentiate into cells that can colonize on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for regeneration of dental tissues. [29] Marie MK *et al.* in their experimental on goat was able to regenerate periodontal tissues around titanium implant.

Regeneration of craniofacial defects:

Stem cells can be useful in the regeneration of bone and to correct large craniofacial defects due to cyst enucleation, tumor resection, and trauma. The closure of a bone defect is commonly carried out with the transfer of tissue, which have disadvantages like, not able to restore the unique function of the lost part, donor site morbidity, accompanied by scarring, infection and loss of function.³⁵ Adipose derived stem cells was used to treat the calvarial defect (120 cm²) of a 7-year-old girl who had severe head injury. Autologous adipose stem cells were extracted from gluteal region along with iliac crest bone graft. Autologous fibrin glue that holds the cells in place was prepared by cryoprecipitation. This successful technique has given new rays of hope that ADSCs can be used for difficult reconstructive procedures.³⁶

Soft tissue reconstruction in the oromaxillofacial region is of paramount importance when there is significant loss of soft tissues during surgery or trauma. Various methods including graft and lap transfer has been tried that produced donor site morbidity. Alhadlaq *et al.* in their experimental studies found human MSCs can turn into adipose cells when they exposed to adipogenic inducing medium. Adipose cells with appropriate shaped scaffold can be used for reconstruction of soft tissues.³⁷

Stem cells isolated from dental pulp has a potential to differentiate into osteoblasts and are a good source for bone formation. Stem cells from oral and maxillofacial region can be combined with bone marrow stem cells to correct larger defects. Oromaxillofacial bone tissue repair with stem cells was done using collagen sponge scaffold and dental pulp stem cells harvested from third molars of the same patient.³⁵

Lagenbach *et al.* in their *in vitro* studies used microspheres (scaffold free tissue construct) to close the critical size bone defects. They found osteogenically differentiated microspheres with outgrowing cells can be used to fill up bone defects. This new procedure has added advantage of permitting the transplantation of more cells and better integrity compared with cell suspensions or gels.³⁷

International Journal of Novel Research in Life Sciences

Vol. 4, Issue 1, pp: (12-18), Month: January – February 2017, Available at: www.noveltyjournals.com

Stem cells isolated from SHED has significantly promoted wound healing in nude mice, proving deciduous teeth can be utilized for the treatment of chronic wounds. This application can be extended into oromaxillofacial region to enhance wound healing.

FUTURE TISSUES:

Future tissues like tissue engineered bone grafts, engineered joints and cranial sutures can be developed with stem cell therapy. A team of professionals including stem cell biologists, molecular biologists, geneticists, polymer and materials scientists, mechanical engineers and clinicians with knowledge of oral and maxillofacial disorders is needed to develop the field of craniofacial tissue engineering. The ability to design anatomically viable and functional bone would have great potential for oromaxillofacial reconstructions of congenital defects, cancer resections, and trauma. The anatomically shaped viable bone grafts like articular condyles can be engineered by using adult mesenchymal stem cells and biomimetic scaffold bioreactor.

Tissue engineered temporo mandibular joint was created by having natural bone building process as an inspiration. Condyle shaped scaffolds were made using decellularized bone with help of digitized clinical images. Stem cells were seeded into the scaffold and placed in a bioreactor chamber containing culture medium. In future this technique can be applied to regenerate other bones in oromaxillofacial region.

III. CONCLUSION

The future dentistry will be more of regenerative based, where patients own cells can be used to treat diseases. Stem cell therapy has got a paramount role as a future treatment modality in dentistry. Regenerative dentistry will have to go in pace with regenerative medicine. On the other hand, stem cells should be differentiated to the appropriate cell types before they can be used clinically, otherwise it might lead to deleterious effects. Determining the role of local conditions such as the type of scaffold and the presence of the microorganisms should be very carefully analyzed. Longer patient follow up is needed to study the life time of regenerated tissue.

REFERENCES

- [1] Stem Cell Information. The National Institutes Of Health Resource For Stem Cell Research <http://Stemcells.Nih.Gov/Info/Media/Promise.Htm>.
- [2] Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol.* 1966; 16(3):381–90.
- [3] Chandramauli PE, S. Manoj kumar, S. Senthil. Stem cells in dentistry A Review. *J Phar. Sci. & Res.* 2012;4(7): 1872-1876
- [4] Egusa H, Schweizer FE, Wang CC, Matsuka Y, Nishimura I. Neuronal differentiation of bone marrow-derived stromal stem cells involves suppression of discordant phenotypes through gene silencing. *J Biol Chem.* 2005;280(25):23691–7.
- [5] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA.* 2000; 97:13625–30.
- [6] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284:143–7.
- [7] Barria E, Mikels A, Haas M. Maintenance and self-renewal of long-term reconstituting hematopoietic stem cells supported by amniotic fluid. *Stem Cells Dev.* 2004;13(5):548–62.
- [8] Prusa AR, Marton E, Rosner M, Bettelheim D, Lubec G, et al. Neurogenic cells in human amniotic fluid. *Am J Obstet Gynecol.* 2004;191(1):309–14.
- [9] De Gemmis P, Lapucci C, Bertelli M, Tognetto A, Fanin E, et al. A real-time PCR approach to evaluate adipogenic potential of amniotic fluid-derived human mesenchymal stem cells. *Stem Cells Dev.* 2006;15(5):719–28

International Journal of Novel Research in Life Sciences

 Vol. 4, Issue 1, pp: (12-18), Month: January – February 2017, Available at: www.noveltyjournals.com

- [10] Hurlbut W, Doerflinger R. Can a morally acceptable way be found to obtain embryonic stem cells? *Origins*. 2004;34(27):429–33.
- [11] Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA*. 1996; 93:705–8.
- [12] Gang EJ, Jeong JA, Hong SH, Hwang SH, Kim SW, et al. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. *Stem Cells*. 2004; 22(4):617–24.
- [13] Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, et al. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet*. 2004; 364:766–70.
- [14] Tocci A, Forte L. Mesenchymal stem cell: use and perspectives. *Hematol J*. 2003;4(2):92–6.
- [15] Stem cell market analysis fact sheet. Available at <http://www.stemcellsummit.com/2007/stem-cellfact-sheet.pdf>. Accessed November 18, 2007.
- [16] . Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* 2000; 97:13625-30.
- [17] . Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 2003; 100:5807-12.
- [18] Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; 364:149-55.
- [19] . Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study. *J Endod* 2008; 34:166-71.
- [20] Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol* 2005; 24:155-65.
- [21] Laughlin MJ. Umbilical cord blood for allogeneic transplantation in children and adults. *Bone Marrow Transplant*. 2001;27(1):1–6.
- [22] . Hurlbut W, Doerflinger R. Can a morally acceptable way be found to obtain embryonic stem cells? *Origins*. 2004;34(27):429–33.
- [23] .Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA*. 1996; 93:705–8.
- [24] Gang EJ, Jeong JA, Hong SH, Hwang SH, Kim SW, et al. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. *Stem Cells*. 2004;22(4):617–24.
- [25] Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, et al. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet*. 2004; 364:766–70.
- [26] Young HE, Black AC Jr. Adult stem cells. *Anat Rec A Discov Mol Cell Evol Biol*. 2004; 276(1):75–102.
- [27] Parker GC, Anastassova-Kristeva M, Broxmeyer HE, Dodge WH, Eisenberg LM, et al. Stem cells: shibboleths of development. *Stem Cells Dev*. 2004; 13(6):579–84.
- [28] Lee N, Thorne T, Losordo DW, Yoon YS. Repair of ischemic heart disease with novel bone marrow-derived multipotent stem cells. *Cell Cycle*. 2005; 4(7):861–4.
- [29] Tocci A, Forte L. Mesenchymal stem cell: use and perspectives. *Hematol J*. 2003;4(2):92–6.
- [30] Stem cell market analysis fact sheet. Available at <http://www.stemcellsummit.com/2007/stem-cellfact-sheet.pdf>. Accessed November 18, 2007.

International Journal of Novel Research in Life Sciences

Vol. 4, Issue 1, pp: (12-18), Month: January – February 2017, Available at: www.noveltyjournals.com

- [31] Pompilio G, Cannata A, Peccatori F, Bertolini F, Nascimbene A, et al. Autologous Peripheral Blood Stem Cell Transplantation for Myocardial Regeneration: A novel strategy for cell collection and surgical injection. *Ann Thorac Surg.* 2004; 78:1808–12.
- [32] Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum.* 2006;54(4):1222–32.
- [33] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002; 13:4279–95.
- [34] Peptan IA, Hong L, Mao JJ. Comparison of osteogenic potentials of visceral and subcutaneous adipose-derived cells of rabbits. *Plast Reconstr Surg.* 2006; 117:1462–70.
- [35] Gimble JM, Guilak F. Differentiation potential of adipose derived adult stem (ADAS) cells. *Curr Top Dev Biol.* 2003; 58:137–60.
- [36] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002; 13:4279–95.
- [37] Schäffler A, Büchler C. Concise review: adipose tissue-derived stromal cells — basic and clinical implications for novel cell-based therapies. *Stem Cells.* 2007 Apr;25(4):818–27.